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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Tiecheng A. Qiao, et al

RANDOM ARRAY OF MICRO-SPHERES FOR THE ANALYSIS OF NUCLEIC ACIDS

RCE of Serial No. 10/036,828

Filed 21 December 2001

Group Art Unit: 1634

Examiner: Betty J. Forman

I hereby certify that this correspondence is being transmitted via facsimile today to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Carol A. Kukuruda

September 21, 2004
Date

Commissioner for Patents
P.O. Box 1450
Alexandria, VA. 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. §1.131

We, Tiecheng A. Qiao, Krishnan Chari, and Douglas L. Vizard, declare that:

Tiecheng Qiao received a degree of Chemistry from the University of Rochester in 1995, and has been employed as a research scientist with Eastman Kodak Company since 1997 in research and development of new products;

Krishnan Chari received a Ph.D. in 1985 from Rensselaer Polytechnic Institute, and is a Senior Principal Scientist in the Research Laboratories of Eastman Kodak Company, where he has been employed for 19 years;

Douglas Vizard received a B.S. in Physics in 1966 from Worcester Polytechnic Institute, and a Ph.D. in Biophysics in 18972 from Penn. State University, and is a Principal Scientist at Eastman Kodak Company, where he has been employed since 1987;

we are co-inventors of the above-captioned patent application; and

we are familiar with the Office Action dated June 21, 2004, and the reference Guire et al., US Patent Application Publication 2003/0073086 A1, published April 12, 2003, and filed October 5, 2001, cited therein.

The claims in the above-identified application in which we are co-inventors, referred to herein as "the pending application," reflect an invention conceived and reduced to practice before the filing of the application by Guire et al., as evidenced by the attached copied notebook pages from the notebook of co-inventor Tiecheng A. Qiao, Notebook BB7408; from the notebook of laboratory assistant Brian Kelly, working under the direction of Tiecheng Qiao, Notebook BB9618; and from the records of co-inventor Douglas Vizard. All work was done in the United States in Rochester, New York.

As shown in the attached copies of pages 131 and 132 of Notebook BB7408, on August 16, 2000, the idea of identifying biological samples by labeling a microarray with one or more optical barcode, contacting the microarray with a target biological sample, and detecting the labeled sample, as claimed in claims 1 and 21 of the pending application, was recorded. Various embodiments of this idea are shown in the figure entitled "Alternative Binding Protein Arrays" dated October 10, 2000, and the figure entitled "Universal Application of Random Bead Array" dated December 21, 2000, both from the notes of Douglas Vizard.

The microspheres, labeled with a colorant, were made by Tiecheng Qiao on February 13, 2001, as recorded on page 138 of Notebook BB7408. Preparation of a microarray using the microspheres, hybridization of the same to a DNA sample, and detection and identification of the sample were done in an experiment on March 9, 2001, as recorded on pages 139-141 of Notebook BB7408, and included a procedure for hybridization of DNA to the colored bead first performed on February 13, 2001, and recorded on page 136 of the same notebook.

Pages 56-58 of Notebook BB9618 show further work on June 26, 2001, wherein an oligonucleotide strand was dissolved, hybridized to a colored bead-labeled strip, conjugated, and imaged according to one method of the invention. This work was performed by Brian Kelley under the direction of Tiecheng Qiao.

Developmental work on the method continued, as evidenced by the October 24, 2001, notes of Douglas Vizard regarding imaging of the microspheres, and the December 7, 2001 image entitled "Spot Size from Pointilliste 96-well Prints," showing spot size and resolution of the image dependent on the number of pixels used in the imaging system, also from Douglas Vizard's notes.

Applicants note that preparation of the pending application was done in parallel with the above developmental work, and was started about June 4, 2001, with the Eastman Kodak Company patent department.

The work reflected in the attached pages provided herewith shows conception and reduction to practice of the claimed invention of the pending application, as set forth at least in independent claims 1 and 21, before the filing date of Guire et al.

The undersigned declares further that all statements made herein of the undersigneds' own knowledge are true and all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____ _____

Tiecheng A. Qiao

Date: _____ _____

Krishnan Chari

Date: _____ _____

Douglas L. Vizard

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. BB7408 PAGE 13

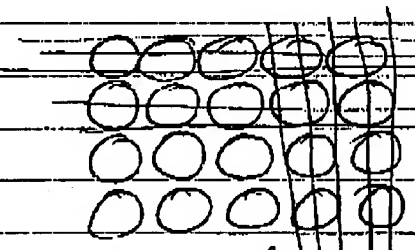
Date 8/16/00

Problem: Radom color coded polymer beads based DNA array/protein array

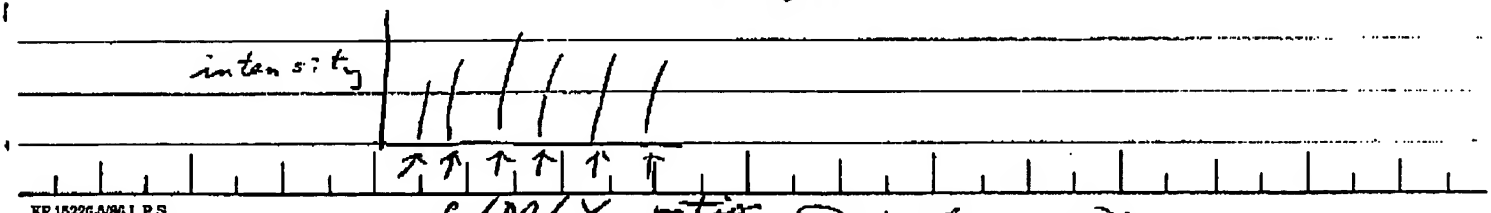
1. polymer beads incorporated w/ various ratio of cyan, magenta, and yellow dyes. The ratio will be used to index each bead for differentiation / barcoding

2. Beads w/ certain ratio dye incorporated are surface derivatized to be able to react w/ DNA / protein
e.g. $-CO_2H$, $-NH_2$, CHO,

3. oligo probes are attached to the surface of dye registered beads, and beads are coated into 2-D array



4. The random coated bead array containing various oligo probes is imaged by a CCD camera and the image is digitized so that the size of pixel is smaller than the diameter of each single bead. The imaging processing algorithm will be used to read the registerd bead individually and the result is summarized based on dye ratio.



KP 1822GA/M&T P.S

Signature

The foregoing disclosed to me on June 27 2001

Witness

PAGE 132
Notebook No. BB7408

RESEARCH / DEVELOPMENT
EASTMAN KODAK COMPANY

Date

Problem:

5 DNA sample is labeldd by conventional PCR meth
w/ fluorescent dye labeled primers, and bybrid
to the beads DNA array.

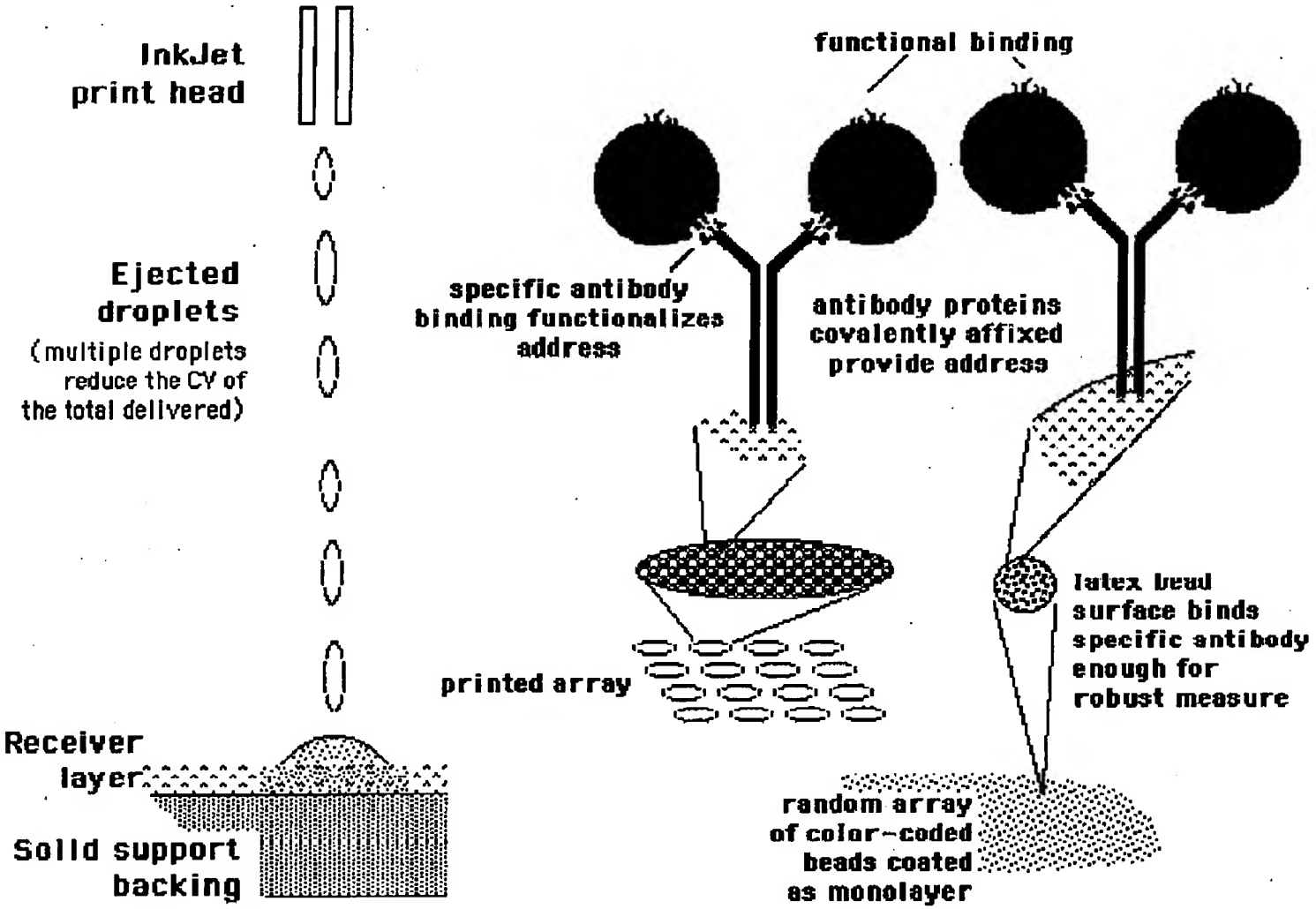
6. The imaging proces by CCD was repeated and
fluorescence signal was analyzed accoraling to dye
ratio registration.

KP 16228-6/88 I. P. S.

Signature

The foregoing disclosed to me on Jan 23 2001

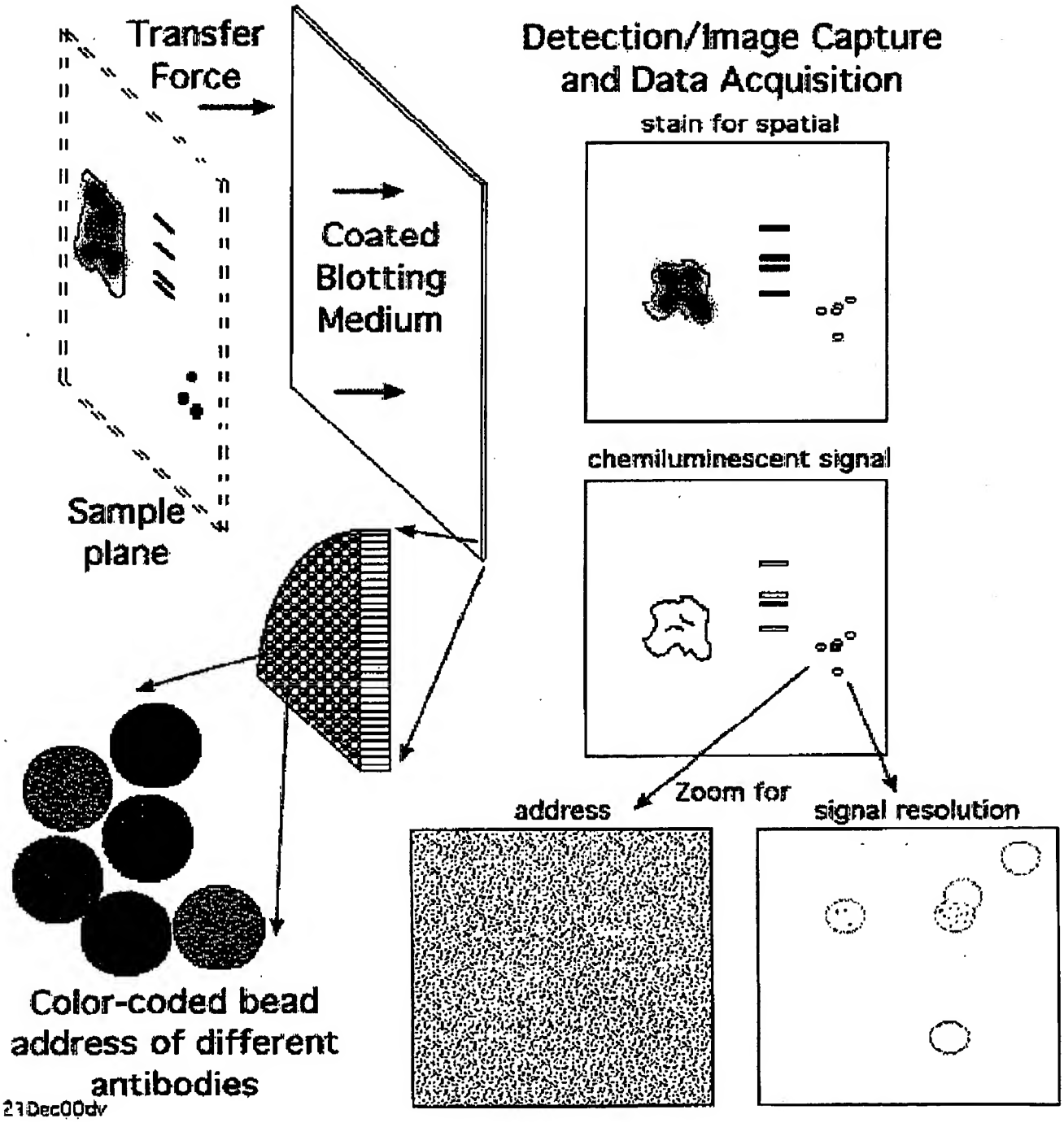
Alternative Protein Binding Arrays



The point of the protein array is to sort proteins or other "molecules" of interest among distinguishable addresses. The addresses may be resolved spatially by either printing or an encoded association (e.g., colored bead). The address is defined by a specific antibody binding. Secondary single-chain antibodies are then engineered to bind to the primary antibodies and present the functional binding for array sorting. Presentation of about 1000 different addresses (which can be changed) enables the application of very powerful sorting methods to the donconvolution of complex populations.

100ct00dv

Universal Application of Random Bead Array



PAGE 138 BB7408

Notebook No.

Date 2/13/01

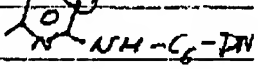
RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

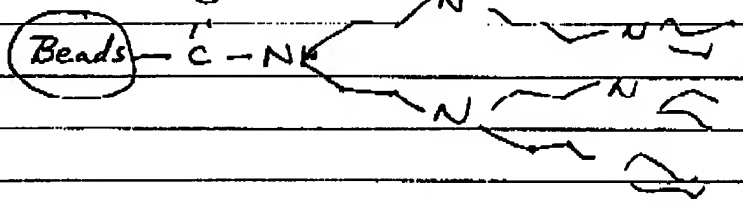
Problem:

Purpose: Hybridization of DNA attached beads to fluorescein modified complementary DNA sample.

A: Beads—C(=O)—NH—C_6—DNA probe.



B:



Both A and B were re-suspended in 500μL 4x SSPE buffer. a concentrate fluorescein labeled DNA sample (123nmol / 123μL = 1 nmol/μL (1mM)) was added to both tubes to make final concentration of fluorescein labeled DNA ~ 2μM.

Tubes were floated in 65°C water bath to cool down to RT (ca 3 hrs)

Spin down samples after hybridization, and wash 3X w/ 4X SSPE buffer

Samples were spread onto microscope slides to visualize fluorescence.

PEI treated sample has much brighter signal than direct coupled DNA beads.

XP 10228-6/86 I. P. S.

Signature

05-29-01

PAGE 136 BB7408 RESEARCH / DEVELOPMENT

Notebook No. _____

EASTMAN KODAK COMPANY

Date 02/13/01

Problem: _____

Purpose: Attachment of 5'-Amino-DNA oligomer to -COOH modified beads (10nm)

DNA: 5'-Amino modified DNA was made by Integrated DNA Technology. 7.1nmol total DNA was re-suspended in 80uL H_2O to give 1nmol/uL stock solution (1).

Coupling: A: direct coupling

0.1g OB1207 was dissolved in 9mL of H_2O to give 1000mM OB1207 stock solution (2)

60mg of cyanuric chloride was dissolved in 80mL acetonitrile to give 4mM stock (3)

2 tubes of 70uL 4% (W/V) beads (ca. 10nmol -COOH groups) was rinse 3X in 0.01M acetate buffer, and re-suspend in 460uL 0.01M acetate buffer pH 5.0 ~~respectively~~

To Tube 1, 20uL DNA stock (1), 2.0uL OB1207 stock (2) were added to make total 200uL, react at RT for 1h w/ agitation. Spin down beads after the completion of reaction and wash 3X w/ 0.01M phosphate buffer pH 7.0. This is DNA attached beads probe 1

KP 15226-5/86 I. P. S.

Signature _____

The foregoing disclosure was ~~read~~ 05-29-01

RESEARCH / DEVELOPMENT Notebook No. BB7408

EASTMAN KODAK COMPANY

Date 3/9/01

Item:

Purpose: Hybridization of DNA attached beads mixed w/ other colored ~~dummy~~ beads.

Both bare bead and EVV dyed bead were labeled w/ DNA on their surface as page 136.

100 µL 4% beads were rinsed three times w/ acetate buffer pH 5.0 and resuspend in 160 µL acetate buffer. 20µL OB1207 stock (0.1g in 4mL H_2O) was added together w/ 20µL 5% PEI (MW 2000). The mixture was agitated at RT for 1 h. The reaction mixture was rinse 3x w. 0.05 M boric acid buffer pH 8.3. and was ready for further DNA attachment.

DNA sample for LDT was resuspended in H_2O to give 1 nmol/µL stock. 40µL of DNA stock was mix w/ 40mL cyanuric chloride (60mg in 80mL acetonitrile) and the total volume was adjusted to 250µL by boric acid buffer pH 8.3, i.e. 5 M. React at RT for 1 h and dialysis against 0.05 M boric buffer pH 8.3 for 3 h at RT.

100µL cyanuric chloride activated DNA was added to PEI modified bead and react at RT for 1 h. Spin and wash 3x w phosphate buffer 2x w 6x SSPE-T buffer.

Signature

The foregoing disclosed to me on 19

Witness

PAGE 140 Notebook No. BB7408

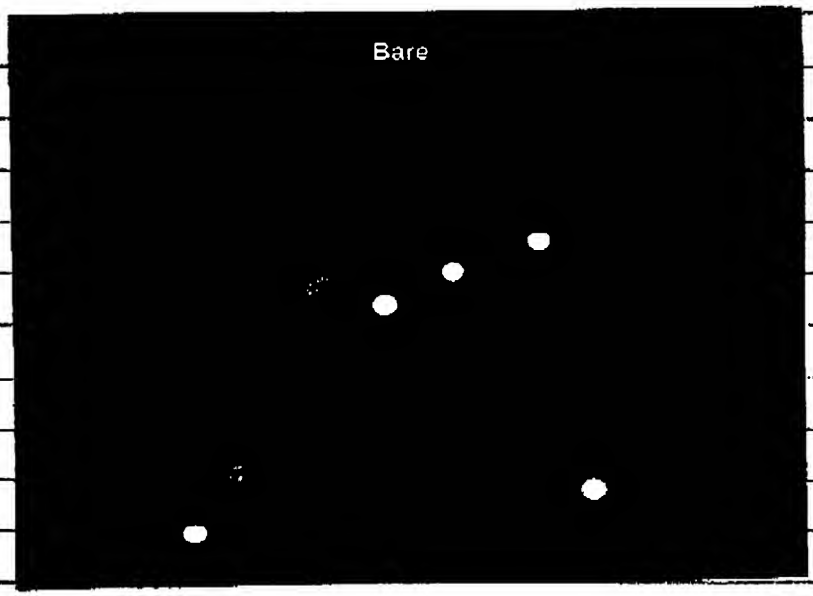
RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Date 3/9/01

Problem:

The DNA modified beads were mixed w/ cyan yellow dyed dummy bead equally and hybridize to fluorescein labeled DNA @ 70°C ~~overnight~~ hunter bead 2min and quenched on ice for 5 min The images were captured under fluorescence microscope in ATD Physical characterization lab by K. Gatien



Bare beads surface was labeled w/ DNA probe and fluoresce under microscope. EVV and cyan dyed beads have no fluorescence, yellow beads glow yellow light for unknown reason

KP 16228-6/88 I. P. S.

Signature

The foregoing disclosed in me on 05-29-01

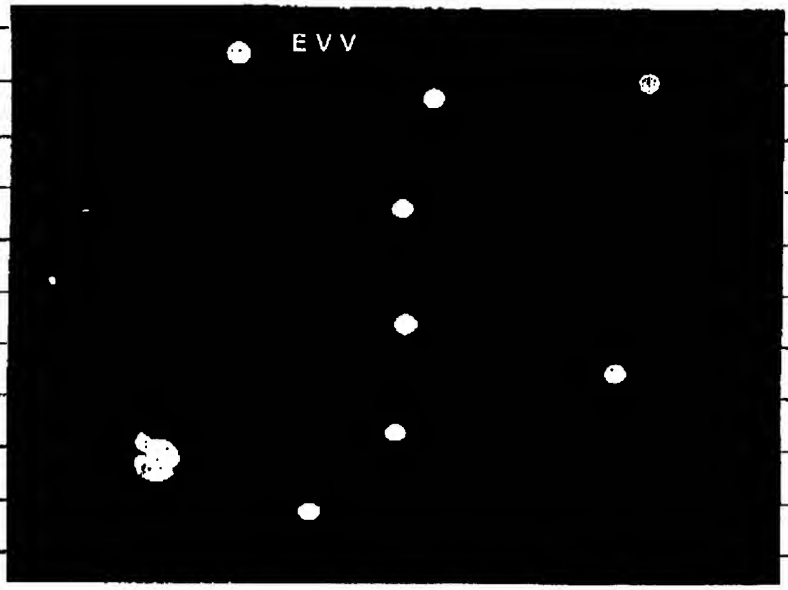
RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. BB7408 PAGE 141

Date

Problem:



EVV beads surface was labeled w/ DNA probe

15225-6/66 L. P. S.

Signature

The foregoing disclosed to me on 19

PAGE 56

Notebook No. BB9618 RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Date 6/26/01

Problem: DNA Hybridization on Polymer beads w/ Chemiluminescent detection

1. A 22-mer oligonucleotide DNA with 5'-biotin labeling, which has complementary sequence to the 22-mer DNA probe, was dissolved in a hybridization solution containing 0.9 M NaCl, 0.06 M NaH_2PO_4, 0.006 M EDTA, and 0.1% SDS, pH7.6 (6XSSPE-SDS) to a final concentration of 2 µM (2 µL stock diluted into 1 mL).
2. A small section of the strip was cut from the main piece with a notch cut in the lower right hand portion while the emulsion side was up. The bead coated strip was hybridized in the hybridization solution (ca. 500 µL) starting at 70°C with a 1 L water bath solution and slowly cooled down to room temperature. Tubes were then placed in an ice bath for 10 min. for further cooling.
3. Following hybridization, the strip was washed in 500 µL of 0.5XSSPE-SDS for 3X with 10 minutes each time.
4. The strip was immersed in 500 µL. Avidin/HRP conjugate solution (1:1000 dilution into PBS Tween 20 buffer, pH 7.4) for 1 hour and washed 3X with 500 µL of dilution buffer 10 min each time.
5. The image was acquired using Olympus BH-2 microscope (Diagnostic Instruments, Inc. SPOT camera, CCD resolution of 1315 x 1033 pixels) with DPlanapo40 UV objective, mercury light source, blue excitation & barrier filters.

7/18/02 CAL

KP 15226-6/00

Signature Brian Keller Witness Celine Lawless

The foregoing disclosed to me on 7/18 20 02

RESEARCH / DEVELOPMENT Notebook No. BB9618

EASTMAN KODAK COMPANY

Date 6/26/01

Problem: Results from p56

Magenta CL (10 min Exp.)

KP 15226-6/00

Signature Brian Hullen

The foregoing disclosed to me on 7/18 2002 Witness Celia Ranken

PAGE 58 BB9618

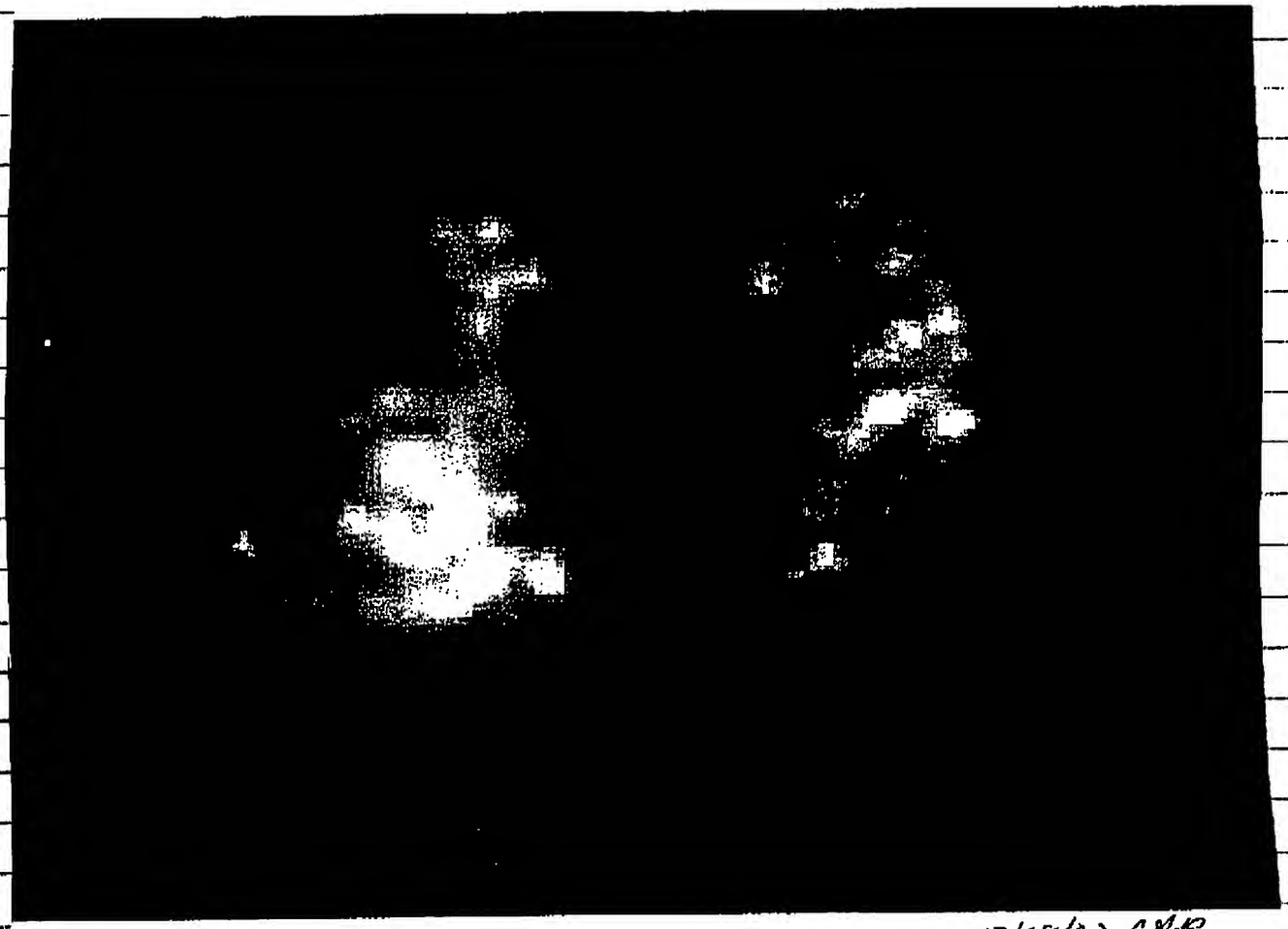
RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No.

Date 6/26/01

Problem: Results from 56



7/18/02 CAR

Yellow CL (Comin Exp)

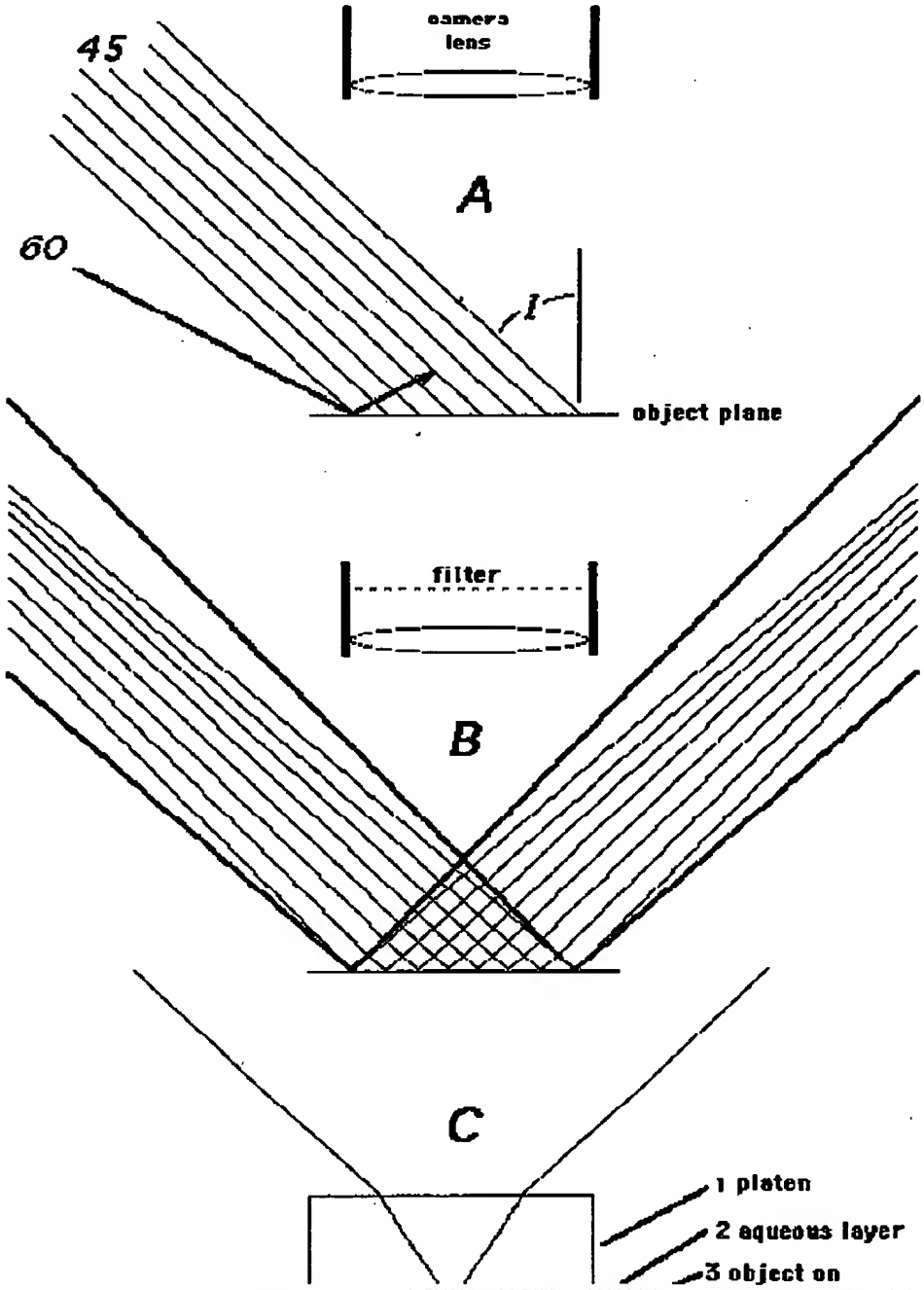
KP 15226-6/00

Signature Brian Killey

The foregoing disclosed to me on 7/18 2002 Witness Eileen Rankin

Imaging/detecting colored beads/fluorochromes/chemiluminescence
24Oct01dv
....prior text must describe colored beads with fluorescent and chemiluminescent reporters, coated on a flat surface as a random dispersion.

The most accommodating approach to obtain image data from a reporting flat object is full-frame image capture. The most important physical aspects of the image capture system is the balance between efficient light collection, optical resolution and effective illumination of the object, and the balanced system must accommodate luminescent, fluorescent and absorbance modes of optical reporting. Further, the system must do so without significant movement the object of interest and the imaging data must support the necessary measures relating to subsequent analysis. The



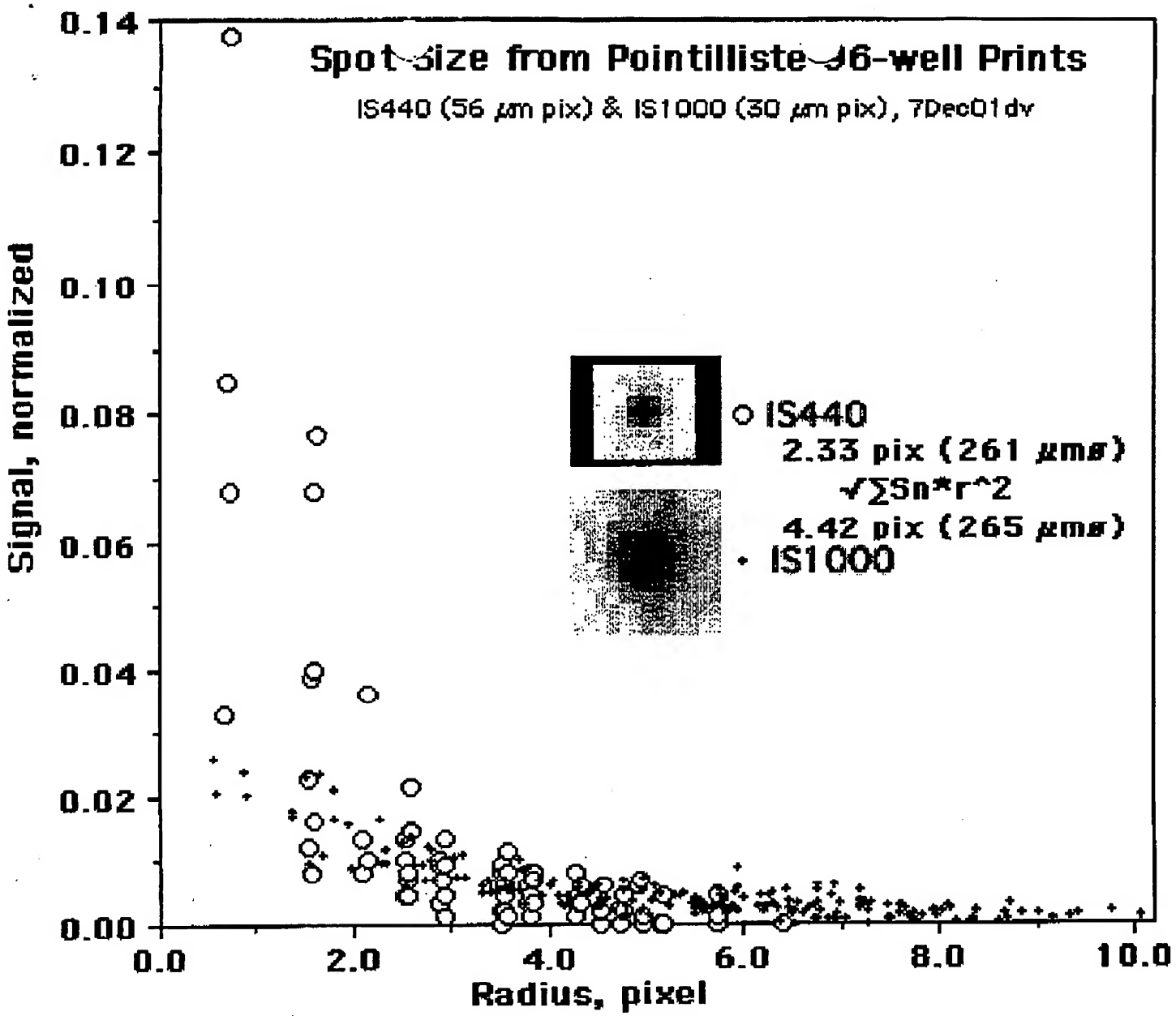
surface (object plane) would involve illumination with parallel light at a 45o angle of incidence (shown as "I" in Figure xA). In practice, an angle of incidence substantially less than 45o increases the possibility that excitation light from a normal reflection will enter the acceptance cone of the lens; sources of normal reflection include stray light from illumination and features in the field of view that deviate from the object plane. An example of a design using a small angle of incidence is that which uses "ring-light" illumination. Another constraint is that the angle of incidence must be less than about 60o, else an increasing amount of excitation light will not interact with the sample due to the total reflection caused by the critical angle of the platen (Figure xC). Any practical system of illumination will include light that is not parallel. It is practical, indeed usual, to illuminate with light that is divergent as illustrated in Figure xB, where the illumination is shown as the finer lines entering from both sides of the object, a common method of epi-illumination used to enhance illumination uniformity. The illustration depicts slightly divergent light (about 18 degrees, or approximately f11), much less divergent than many commonly used methods such fiber illumination. The epi-illumination shown in Figure xB is preferred, since the extreme angles of incidence (and reflectance shown as bold lines) minimize the possibility of stray light entering the acceptance cone of the lens or critically reflecting from a planar surface.

A further important system design solution is embodied in Figure xC. The platen composition is a controlled optical surface, and as such may be constructed to minimize haze, autofluorescence and reflectance. Note that in this system design, the platen is the only material with which the excitation light has the opportunity to interact. Preferred composition of the platen is optical acrylic (UV transparent), whose sensor/illumination side is coated with an anti-reflective layer, and whose sample/object side is coated with an anti-abrasion layer (hard-coat). Other compositions (such as fused silica) are acceptable, but will manifest some performance decrement (transmit less light). As described above, the aqueous layer (ubiquitous to biological/biochemical systems) optically couples the sample surface to the platen and the object (the bead monolayer) is coated onto a flexible support having a mirror surface. The vast majority of the excitation light, which does not interact with the object beads, is simply reflected out of the system, away from the lens. To summarize, in this preferred epi-illumination system for fluorescent excitation, the excitation light interacts only with a controlled optical component, a thin aqueous solution and a target bead before the light has a significant probability of entering the acceptance cone of the lens. Consequently, the lens system need only discriminate excitation from emitted light that emanates from the target bead. The discrimination of excitation from emitted light is commonly practiced in fluorescent imaging/detection, commonly using an interference filter (see Figure xB). Such filters can be very costly and the capability of the filter to efficiently discriminate excitation and emitted light is often the chief limitation of detection sensitivity. In this preferred system design, the requirement of the filter to discriminate is reduced approximately 1000-fold compared to many fluorescent detection systems.

The chief requirements of absorption imaging/detection (generally called colorimetry) involve spectral resolution and photometry. The photometric precision of the system described above is more than 10-fold better than the basic system requirements for resolving thousands of "colors". The application requirement is similar to resolving/deconvoluting R, G, B and combinations/levels thereof. The fundamental method of color resolution involves measurement of the relative absorption characteristics of an object with differing wavelengths of light; the relative differences are tabulated to functionally define categories of dye combinations and levels. The dyes chosen must not interfere with other reporting optical elements, such as fluorochromes. One way to minimize anticipated interference is to choose "color" dyes whose absorbance spectrum is higher than any excitation/emission spectrum of the reporting fluorochromes.

Given a sufficient photometric precision, the number of colors that may be resolved (functionally defined) will depend upon the management of illumination and detection of light. Two fundamentally different methods of light management for the purpose of color resolution are either spectrally resolving "white" light before or after the light interacts with an object. The above system design requires that both methods be invoked for the sake of fluorescent imaging. The preferred design solution that is suitable for both absorption and fluorescent modes of imaging detection is to manage the spectral resolution of the illumination. Therefore, the illumination is

managed by a light source and monochrometer, having sufficient power and resolution to accommodate both modes of imaging. The solution is cost effective because the multi-filter design and implementation that would accommodate the excitation of multiple fluorescent probes and absorption of multiple colored dyes would easily exceed the cost of monochromator. Since the spectral excitation/absorption of the organic fluorochromes and dyes are relatively broad, a spectral resolution of only about 20 nm will suffice and the delivered illumination energy will be correspondingly higher than that associated with a "high resolution" monochromater (typically < 2 nm). That the delivered energy will suffice for fluorescent imaging is assured by the sensitivity of the camera which is designed for luminescent imaging.


Spot Size from Pointilliste 96-well Prints
IS440 (56 µm pix) & IS1000 (30 µm pix), 7Dec01dv
Signal, normalized
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.0
2.0
4.0
6.0
8.0
10.0
Radius, pixel
IS440
2.33 pix (261 µmσ)
√∑Sn*r^2
4.42 pix (265 µmσ)
IS1000